d) determining the difference in determined ΔT_m between overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid.

REMARKS

Claims 1-16 are pending in the instant application. Claims 1-16 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. A version of the claims indicating the amendments being made in this response is attached under the heading "Version Showing Changes Made." A version of the claims after entry of these amendments is attached under the heading "Appendix of Pending Claims."

The Invention

The instant invention relates to methods for screening for and identifying sequence alterations in nucleic acids. In these methods a number of nucleic acid probes are hybridized to completely complementary regions of a control nucleic acid as well as to a target nucleic acid that may or may not be completely complementary. By measuring the dissociation temperatures of the various probes with either the control or the target nucleic acid, it is possible to determine if differences are present between the control and target nucleic acids. Furthermore, by using overlapping probes in these methods it is possible to use differences in dissociation temperature to indicate the exact location and identity of any differences.

In one method, a plurality of overlapping probes are used to identify sequence alterations. These probes, when taken together, are an exact complement to a control nucleic acid sequence. The probes are individually hybridized to a target sequence and the dissociation temperature, T_m, is measured for each individual probe bound to the target. These T_ms are then compared to the T_ms of the individual probes when bound to the control sequence. The difference between the

two T_ms for any one probe is termed the ΔT_m. A change in the dissociation temperature between the probe/control pair and the probe/target pair is indicative of a difference in sequence between the control and the target sequences. Because of the overlapping nature of the probes, there will always be a second probe that hybridizes at the site of the difference in sequence between control and the target sequences. By comparing the ΔT_m of the first probe and the ΔT_m of the second probe you are able to determine the $\Delta\Delta T_m$. Because every sequence alteration will cause a distinctive change in the ΔT_m, the $\Delta\Delta T_m$ can be used to indicate the location and identity of the nucleotide difference between the control and target sequences.

In another method, a plurality of nucleic acid probes, again complementary to regions of a control sequence, are hybridized to a target sequence and T_ms are measured. The difference between this method and the one described above is that a first set of nucleic acid probes complementary to regions of the control sequence separated by one or more nucleotides are used along with at least a second set of nucleic acid probes complementary to regions of the control sequence that are also separated by one or more nucleotides. Furthermore, the regions complementary to the secondary set (or sets) of probes include the nucleic acids separating the first set of probes as well as overlapping with the regions complementary to the first set of probes. By determining the ΔT_ms and $\Delta\Delta T_m$s, as described above, one can find the location and identity of alterations between the control and target sequences.

Claims 1-16.

The Examiner asserts that Claims 1-16 are vague and indefinite because it is unclear whether or not the plurality of nucleic acid probes are complementary to the different overlapping regions of the target nucleic acid, since in figure 1 it appears that the plurality of probes hybridized to the different overlapping regions of the target nucleic acid.

Figure 1 contains two parts, a schematic of how a plurality of overlapping nucleic acid probes could hybridize to a target sequence and a presentation of actual data where target nucleic acids contain a single mismatch in the region bounded by probes A and B as compared to the

control sequence. Figure 1 is not intended to convey that each of the plurality of nucleic acid probes are exact complements to regions of the target nucleic acid.

While the methods of the instant application are applicable to screening for and identifying nucleic acid alterations in nearly identical control and target sequences, they are equally applicable to divergent control and target sequences. By measuring the T_ms of probe/target pairs and comparing them to probe/control pairs one can screen for and identify nucleic acid alterations in targets that have little or no complementarity to the probes.

Because there is no need to identify any length of complementarity between the probes and the target sequence, no amendment to the claims is necessary. Applicants respectfully request withdrawal of the rejection.

Step c) of Claims 1 and 6

The Examiner also asserts that step c) of Claims 1 and 6 are not clear in explaining how ΔT_m is determined. Specifically, the Examiner asks whether the terminology means that the ΔT_m is separately determined between two probes in the target nucleic acids and between the same two probes as hybridized to the target nucleic acid on the control nucleic acid, or the ΔT_m is determined between one probe on the target nucleic acid and the same probe as hybridized to the target nucleic acid on the control nucleic acid.

Step c) of Claims 1 and 6 has been amended to make clear that ΔT_m is determined, as discussed above, by comparing an individual probe's dissociation temperature from the control sequence and its dissociation temperature from the target sequence. Accordingly each probe will have its own ΔT_m. In light of these remarks and the amendment to the Claims, the Applicants respectfully request withdrawal of this rejection.

Claims 12-16

Finally the Examiner asserts that Claims 12-16 are vague and indefinite because Claim 12 is not clear in explaining how ΔT_m is determined. Specifically, the Examiner asks whether the

terminology means that the ΔT_m is determined between one probe of said first set of probes and one probe of second set of probes.

Claims 1 and 6 have been amended to more clearly explain how ΔT_m is determined. Specifically, ΔT_m is determined for a specific probe by comparing the dissociation temperature of that probe and a control sequence that it is completely complementary to and the dissociation temperature of that probe and the target sequence. Claim 6 requires, at minimum, determining the ΔT_m for 2 individual probes. Claim 12 adds the further limitation to the method of Claim 6 that ΔT_ms are determined for at least two probes of the first set and at least one probe of a second set, wherein the probe or probes of the second set overlaps the two or more probes of the first set. Therefore Claim 12 requires, at minimum, determining the ΔT_ms for 3 individual probes.

In light of the above remarks and the amendment to Claims 1 and 6, the Applicants respectfully request withdrawal of this rejection.

CONCLUSION

Applicants respectfully submit that the above amendments and remarks overcome all of the rejections under 35 U.S.C. 112, second paragraph, and therefore all claims are now in condition for allowance and an early notification of such is solicited. If, upon review, the Examiner feels there are additional outstanding issues, the Examiner is invited to call the undersigned attorney

Respectfully submitted,

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VERSION TO SHOW CHANGES MADE

1. (Amended) A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein said probes are complementary to different overlapping regions of said control nucleic acid;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

c) determining for each of said at least two probes the difference between the T_m of [from] said target nucleic acid each of said at least two probes and the T_m from said control nucleic acid [(]ΔT_m[)] for each of said at least two probes; and

d) determining the difference in determined ΔT_m between overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid.

6. (Amended) A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein a first set of probes is complementary to regions of said control nucleic acid separated by one or more nucleotides and at least a second set of probes is complementary to regions of said control separated by one or more nucleotides, wherein the regions complementary to said second set of probes include the nucleic acids separating the first set of probes and are overlapping with the regions complementary to said first set of probes;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

c) determining for each of said at least two overlapping probes the difference between the T_m of [from] said target nucleic acid and each of said at least two overlapping probes and the T_m of [from] said control nucleic acid and each of said at least two overlapping probes thus determining the [(]ΔT_m[)] for each of said at least two overlapping probes; and

d) determining the difference in determined ΔT_m between overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid.